**Methods:** Osteoblasts were isolated from sclerotic (SC) or non-sclerotic (NSC) zones of OA subchondral bone of five men. After 28 days, osteoblasts in culture were surrounded by a newly synthesized matrix and formed a strong membrane. This osteoblasts-containing membrane was then placed onto a Biopress Flexercell plate and submitted to compression (10%) for 4 hours at the frequency of 1 Hz. The expression of integrin α5, αV, β1, β3, CD44 and connexin43 (CX 43) was evaluated by real time RT-PCR

**Results:** In the absence of mechanical stimuli, integrin α5, αV, β1, β3, and CD44 mRNA levels were similar in NSC and SC osteoblasts. Integrin α5 and β1 expression was 10 fold more elevated than αV and β3. CX 43 mRNA levels were significantly lower (−30%) in SC osteoblasts than in NSC osteoblasts ($p < 0.01$). Compression (4h, 10%, 1 Hz) stimulated integrin α5 (1.42-fold), αV (1.37-fold) and β3 (2.27-fold) expression in NSC osteoblasts ($p < 0.01$) but didn't significantly alter integrin β1 or CX 43 expression. In SC osteoblasts, only integrin β3 gene expression was increased by compression ($p < 0.001$), while integrin α5, αV or β1 genes were not modified and CX 43 expression decreased (0.60-fold, $p < 0.001$). CD44 expression was increased by compression similarly both in NSC and SC osteoblasts (1.4-fold, $p < 0.01$).

**Conclusions:** CX 43 plays a major role in osteoblast mechanotransduction. It is less expressed by SC than NSC osteoblasts. Further, CX 43 is decreased by compression in SC osteoblasts. This downregulation of CX 43 could be responsible for the decrease in mechanosensitivity of SC osteoblasts. This finding offers a new perspective of research to explain the role play by mechanical stimuli in OA pathogenesis.

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### CONTINUOUS STRAIN STIMULUS FOR THE DEVELOPMENT OF OSTEOARTHRITIS

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**Purpose:** The subchondral bone is a thin layer of specialized bone covered by articular cartilage. It is widely believed that subchondral stiffening is a primary event in osteoarthritis (OA). How and why subchondral bone stiffens has hitherto been unclear. In this study is followed the hypothesis that strain-levels above genetically determined thresholds stimulate bone-growth. This is supported by a previous study, in which it was shown that the presence of the osteochondral junction itself significantly amplifies the strain in the subchondral bone, by as much as 42 times, over the strains which arise when there is no interfacial region present, i.e. when there are not two dissimilar materials (cartilage / subchondral bone) present. One major issue with this approach is Young's hypothesis, which states that whenever the stiffness of a material increases, the strain will decrease, given a constant stress (following Hooke's Law). Thus, as the bone stiffens, it is expected that the strain will decrease (for a given load through the joint); thus the strain stimulus dies off and the bone growth becomes a severely self-limiting process, never reaching a stage where the significant subchondral stiffening of OA might occur. The purpose of the present study is to address this issue.

**Methods:** Computerised tomography (CT) scans were taken of the hip and knee joints (177–266 slices of 1.25 mm). Voxel based image analysis and visualisation software (Volume Graphics) was used for image segmentation and extraction. Images were further edited and refined using Geomagic Studio 9 software, in order to produce regular and consistent meshes. The data was imported into the ABAQUS 6.10-EF1 Finite Element software. Distinct regions within the model were assigned the material properties of cartilage, and cancellous, cortical and subchondral bone. Physiological loading was applied. The stiffness of the subchondral bone was increased in successive models and the response was analysed. A number of idealised geometries were also analysed.

**Results:** It was found that, as the subchondral bone stiffens, complex changes take place in stress and strain in the vicinity of the osteochondral junction. Whilst the vertical stress remains largely unchanged, the components of stress tangential to the osteochondral junction increase in magnitude, by up to 500%. Crucially, it is observed that the strains do not reduce significantly, even when the subchondral bone is stiffened by 2500%, close to its theoretically maximum possible value. Interestingly, it was also shown that changes to the material properties of the cortical and cancellous bone did not significantly alter the trends observed.

**Conclusions:** When subchondral bone stiffens, the strain does not nearly drop by an amount which would be required for relatively rapid stimulus die-off. The study provides the evidence that the stimulus for bone growth remains, even in stiffened subchondral bone, and that it is a progressive stimulus. As the subchondral bone continually stiffens, the stress in the overlying articular cartilage increases, in turn making articular cartilage more vulnerable to degradation, and so leading to OA.

## Meniscus, Muscle, Tendon & Ligament Biology

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### HISTOPATHOLOGY OF THE HUMAN ANTERIOR CRUCIATE LIGAMENT IN AGING AND OSTEOARTHRITIS

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**Purpose:** The development and patterns of spontaneous aging-related changes in the anterior cruciate ligament (ACL) and their relationship to articular cartilage degeneration are not well characterized. The aim of this study was to investigate the types and temporal sequence of aging-related ACL changes and establish the correlation with cartilage lesion patterns at all stages of OA development in human knee joints without prior joint trauma.

**Methods:** Human knee joints (n = 120; 65 donors; age 23–92) were obtained at autopsy. Articular cartilage was graded macroscopically using a modified Outerbridge scoring system and the ICRS knee map. ACL was graded macroscopically and histologically. The following parameters were analyzed: collagen fiber organization, cellularity and cell arrangements, chondroid metaplasia, mucoid degeneration, cystic changes and calcification. Inflammation surrounding the ACL was assessed separately.

**Results:** Histological ACL substance scores and synovial sheath inflammation scores increased with aging. Collagen fiber disorganization was the earliest and most prevalent change. Cell density decreased with donor age but in degenerated ACL cellularity increased. These cells were not aligned with collagen fibers and cell arrangements represented islands with large numbers of cells interspersed within hypocellular areas. The severity of mucoid degeneration and chondroid metaplasia in the ACL increased with development of cartilage lesions. A correlation between ACL and cartilage degeneration was observed, especially in the medial compartment of the knee joint. In normal ACL, MMP-3 expression declined with aging. However, in ACL from knees with cartilage degeneration, MMP-3 expression increased. In normal ACL, some CD45 positive cells were present only in perivascular areas in the ligament sheath. In ACL with inflammatory changes, CD45 positive cells were also found between collagen fibers, regardless of the presence of cartilage degeneration. Calcium deposition in ACL is a rare and the latest event in ACL degeneration.

**Conclusions:** ACL involvement is always observed in knees with cartilage damage and ACL degeneration can precede the onset of cartilage lesions. The pathogenic process involves a marked phenotypic shift in ligament cells with an abnormal chondroid differentiation. We propose a temporal sequence of histopathological changes. The earliest changes are collagen fiber disorganization and mucoid degeneration. Inflammation, chondroid metaplasia and cystic changes increase with the presence of cartilage degradation. Calcium deposition is a late and rare event. Thus, cellular changes within the ACL might contribute to aging-related onset and progression of cartilage degradation and OA pathogenesis.